

INTERIM PROGRESS SUMMARY

to

THE COUNCIL FOR TOBACCO RESEARCH--USA, INC.

on

CHARACTERIZATION OF ANIMAL EXPOSURE SYSTEMS

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INTRODUCTION

This memorandum summarizes recent progress on the project to characterize animal exposure devices. Progress for the period June 1974 to January 1975 is summarized. Only abbreviated discussions of experimental results, conclusions, and the current status of the project are presented. Detailed discussions are not included as they are more appropriately presented in open literature publications and an annual progress report will be submitted in June 1975.

CURRENT STATUS

The primary objectives of the project are to establish a battery of tests for evaluating devices for exposing animals to whole tobacco smoke, to apply these tests to devices of interest to the Council for Tobacco Research, and to formulate recommendations for elimination of discovered shortcomings in the devices. Initial efforts were directed at developing methodologies needed to test the devices. This effort is continuing at a reduced level. Emphasis at the present time is on applying available tests to evaluate the Walton-Horizontal, Lorillard LACS II, and Process and Instruments SEM I exposure systems. A number of shortcomings in these systems have been discovered to date. Means for eliminating shortcomings have been devised and implemented in some cases.

Studies on the Walton Horizontal smoking machine have been extensive and have included an in-depth evaluation of the operational features of the machine and an investigation of the chemical properties of smoke offered to animals with the device. Chemical studies include assessment of smoke uniformity in the exposure chamber, losses of smoke from the exposure chamber, and the effect of smoke aging and animal interaction on the concentration and composition of smoke offered to animals with the device. Initial studies on smoke dose received by mice have been completed and more extensive studies are to begin in the near future. Methodology for determining the particle size distribution of smokes in animal exposure systems was recently developed. Initial application of the methodology has been made to the Walton system. Completed studies on the system are being prepared for open literature publication.

Work on the Lorillard LACS II exposure system has been restricted to an extensive operational evaluation and preliminary study of the chemical properties of smoke offered with the system. Complete evaluation of the system has been delayed because of the number and severity of maintenance problems that have been encountered. Although the basic design of the mechanical and electronic components of the system appear adequate, insufficient care was taken in construction of elec-

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tronic circuits that control operation of the system. Continual failure of the electronic circuits has occurred, and a major effort has been required to locate and repair faulty circuits. The system is presently inoperative, and a number of electronic circuits are being reconstructed.

A prototype model of the Process & Instruments (P&I) SEM I exposure system was received during this report period and an extensive operational evaluation was made. Several shortcomings of the system were discovered and design changes were implemented. The design changes are being incorporated in a new model of the system which is presently under construction by P&I. Studies to define the chemical properties of smoke offered by the prototype system are currently under way.

ACTIVITIES FOR THE PERIOD JUNE 1974 TO JANUARY 1975

A. Operational Characterization and Design Improvements in the P&I SEM I System.

1. The electronic timing circuits which control operation of the smoke distribution valve did not permit accurate control of the exposure time. An *improved timing circuit was devised* and recommended to the manufacturer for inclusion in the final model of the system.
2. A *temperature rise of from 65-70°C was found to occur in the vicinity of cigarettes during smoke generation.* A temperature rise of this magnitude is not acceptable because of resulting changes in puff volume and because the chemical composition of the smoke may be altered. *The temperature rise was reduced to 25-30°C by changing the design of the side-stream smoke hood and the air flow system.* Further reduction in the temperature rise will require major design changes if chemical studies reveal that the smoke composition is affected.
3. *The relative humidity of air in the dome area of the system decreases to less than 10% when several loads of cigarettes are smoked.* Because of the possible effects of the low humidity on the burning rate of cigarettes, the chemical composition of the smoke, and deposition of smoke particulates in the animals, efforts are being made to humidify the dome supply air to 60% relative humidity at ambient temperatures. *A device for injection of water into the air supply has been designed and built.* The device is currently undergoing tests.
4. *Fluctuations in dome pressure were encountered when the eject and loader assemblies operated.* The fluctuations produced a change in the puff volume of from 3-5 ml. *A ballast tank installed on the supply air system was found to reduce the pressure fluctuations to an insignificant level.* The ballast tank will be incorporated into the final design of the system.
5. Operation and calibration of the SEM I is easy and uncomplicated. In about three months of heavy usage, *no major mechanical breakdowns, component failures, or other maintenance problems have been encountered.*

6. Measurements of the sidestream smoke concentration in the dome area were made using nicotine and carbon monoxide as smoke indicators. Based on these measurements, *contamination of the mainstream smoke with sidestream smoke is less than 0.5%.*
7. *The static burn rate of cigarettes in the machine under operating conditions was measured. This study showed that the burn rate is not affected by the recommended air flow rate through the dome (350 cfm).*
8. A number of minor design changes based on the initial evaluation of the prototype model were made to the manufacturer in a letter of January 2, 1975.

B. Chemical Properties of Smoke from the P&I SEM I System.

1. Assessment of the quantity and chemical composition of smoke generated and delivered by the prototype system is currently being made. Initial studies deal with the total particulate matter, nicotine, tar, and water deliveries of cigarettes smoked on the system. High resolution gas chromatographic methods for smoke particulates and gas phase are being applied to investigate smoke composition.

C. Operational Studies on the Walton-Horizontal Smoking Machine.

1. Measurement of the static burn rates of cigarettes showed that *the machine would not smoke three cigarettes as designed.* Air venting from the puffing dome flows over the cigarettes and causes the static burn rate to increase so that the cigarettes are consumed with less than the standard number of puffs. *A solenoid valve was installed in the puff air supply to eliminate the problem.*
2. *The procedure for calibration of puff volumes with the machine has been simplified.* Studies showed that the puff volume is not significantly affected by the resistance of the cigarette, eliminating the need for a separate calibration with each type cigarette smoked on the machine.

D. Chemical and Physical Properties of Smoke Presented to Animals with the Walton-Horizontal Smoking Machine.

1. The possibility of changes in smoke composition as the smoke stands in the exposure chamber was investigated using two high-resolution gas chromatographic profiling methods. These methods permit visualization of approximately 250 of the major organic components in the gas and particulate fractions of smoke. Profiles for smoke aged for 30 seconds were almost identical to fresh smoke indicating that *very little or no compositional changes are occurring in the Walton exposure chamber during exposures up to 30 seconds.*
2. Analyses were made for nicotine, nitric oxide, and neophytadiene in the Walton exposure chamber to determine the *effect of aging on these important smoke components.* Only with nitric oxide was aging shown to produce a change in the component concentration. *With nitric oxide, the concentration decrease due to reaction with oxygen was very small--the concentration decreased less than 10% in a 30 second exposure.*
3. The high resolution chromatographic profiling methods were used to establish the effect of animal interaction with smoke in the Walton exposure chamber. Appreciable interaction was found. Twenty mice cause a

general overall decrease in the gas and particulate phase concentration. In addition, several components in the gas phase are affected more than other components resulting in compositional changes. During a 30 second exposure, the overall organic gas phase concentration decreases by nearly a factor of two. One peak in the gas phase profile almost completely disappears and others are affected to a lesser extent. *Animal interaction with smoke in the Walton exposure chamber is potentially a serious problem and should be taken into account in using the system for biological studies.*

4. Analysis of nitric oxide in the Walton exposure chamber during exposures of 20 mice showed that the mice remove nitric oxide from the chamber at an appreciable rate and therefore deplete the chamber of this important smoke constituent. *The nitric oxide concentration is depleted by approximately a factor of two during a 30 second exposure of 20 mice.*
5. Application of newly developed methodology for particle size measurements have been made to determine the effect of smoke aging on the particle size of the smoke aerosol in the Walton exposure chamber. *Initial results show that the particle size is increasing rapidly during a 30 second exposure; the particle size approximately doubles during a 30 second exposure. Studies on the effect of animals on the particle size are currently underway.*
6. Comparisons were made of the composition of smoke generated by the Walton smoking machine and the Phipps and Bird analytical smoking machine. The high resolution chromatographic profiling methods were used for this comparison. This study showed that *smoke generated by the Walton duplicates smoke generated under standard smoking conditions. They also suggest that positive puffing has very little effect on smoke composition.*
7. *Continual use of the Walton machine without cleaning results in build-up of smoke particulates on the internal surfaces of the exposure chamber. Studies were made to establish how frequently the exposure chamber should be cleaned to avoid exposing animals to the gas phase constituents in equilibrium with the particulates deposited on the walls of the chamber. Analysis of the gas phase from the exposure chamber for organic constituents showed very low levels of smoke components after 40 cigarettes had been smoked on the machine. Once-a-day cleaning of the exposure chamber is sufficient to prevent exposure to components in equilibrium with deposited particulates.*
8. Initial dosimetry experiments with mice were performed during the first contract year on the Walton smoking machine. Extensive studies have been planned which will be a collaborative effort of this laboratory and Microbiological Associates. This work will begin with final approval from CTR.

E. Maintenance Problems with the Lorillard LACS II Exposure System.

1. Numerous maintenance problems have arisen in attempting to perform an evaluation of the Lorillard LACS II system. *The main cause for the problems is poor construction of electronic control circuits in the instrument. During this report period, over 25 faulty solder joints have been repaired, and approximately 20 integrated circuits and 17 transistors have failed and have been replaced. The entire electronic circuits of the system are currently being checked and some circuits are being rebuilt. Thorough evaluation of the system is not possible until the electronic circuits are operating properly.*

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2. The quad-valve assembly on the system is inoperative. Lorillard Research has been informed of the mechanical failure of the valve, and is presently redesigning a new valve.

F. Preliminary Studies of Smoke Delivered by the Lorillard LACS II.

1. Measurements were made on the age of the smoke delivered by the LACS II using a thermal conductivity monitor developed during contract year one. These measurements showed that *the smoke is less than 5 seconds old when it reaches the animal exposure unit* (all of the smoke is less than 5 sec. old and 50% of the smoke is less than 2.5 seconds old).
2. Studies on the quantity of smoke delivered by the LACS II show that the smoke is offered to all animals at the same concentration. These studies were made using nicotine as a monitor of smoke concentration.
3. Losses of smoke particulates in connecting tubing used with the LACS II are very small and do not present a problem. Losses in the quad and shuttle valves have not yet been established.
4. The temperature in the vicinity of cigarettes smoked on the LACS II has been measured. It was found that the maximum temperature is 50°C which may have an effect on the chemical composition of smoke generated by the system.

G. Methodological Development and Exposure Concepts

1. A method has been developed for measurement of the particle size distribution of smoke particulates in exposure systems. The method involves encapsulation of smoke particles in a polymer bead and examination by scanning electron microscopy. Initial application of the method has been made to study smoke aging in the Walton Horizontal smoking machine.
2. Exploratory studies have been made on application of *Fourier transform infrared spectroscopy* to study compositional changes of smoke in animal exposure systems. Present indications are that the technique can be used to assess the possibility of very rapid chemical changes which occur within the first few seconds after smoke is generated. Techniques for detecting very rapid chemical changes are not now available.
3. A monitor for continuous measurement of the smoke particulate concentration in exposure systems is under development. Both laser light scattering and light absorption are being considered. A device is needed for particulate monitoring to establish smoke uniformity and concentration.
4. In smoke exposure systems, different smoke generation methods are used. Studies are underway to establish if and how the smoke generation method affects the delivery of the cigarette or the composition of the smoke. The following smoke generation variables are under study: horizontal vs vertical cigarette placement, restrictive vs free smoking, and positive vs negative puffing. Results obtained to date suggest that small but discernable differences in cigarette delivery result from the different smoke generation methods, but that compositional differences in the smoke are not evident.

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